ARTIFICIAL PARTHENOGENESIS IN THE SILKWORM.

VERNON L. KELLOGG.

I may be pardoned, because of the brevity of this paper, for recalling attention to a subject that seems (but is not) pretty well worn. Really only three men (Tichomiroff, Verson and Quajat) have contributed the data of observation and experiment which have furnished the literature of parthenogenesis with such a host of fleeting references that it must seem to the casual reader as if silkworm parthenogenesis had been investigated only less than that of the sea-urchins. As a matter of fact it has been investigated (the work described in the present notes included) but little.

In a clutch of unfertilized eggs oviposited by a virgin silkworm moth (*Bombyx mori*) almost always a small number of eggs begins development. This development extends to the formation of the embryonic envelops and sometimes farther, and is clearly indicated to the observer by the change in color of the egg from yellow to cherry or through cherry to gray. Non-developing eggs remain yellow and, after a while, collapse. Eggs which begin to develop either persist in spherical shape, which indicates persisting life, or collapse, which means death. The development of unfertilized eggs rarely proceeds, without artificial stimulus, beyond a very early embryonic stage. In fully 500 clutches or broods of unfertilized eggs (from confined females from isolated cocoons) under observation, not a single egg gave up its larva, although an average of about seven or eight per centum of the eggs began to develop.

Although this parthenogenetic development always ceases and the embryo dies before reaching hatching stage, much difference in vitality or duration of life of the egg (strictly, embryo) is noticeable. Some of the developing eggs collapse within a few days, some in a few weeks, while a few persist for several months. (The normal egg stage, *i. e.*, time from egg laying to hatching of larvæ in the silkworm univoltin races, is about nine months.)

There is also to be noted a difference among races in the proportion of unfertilized eggs which begin to develop. Among a dozen races in our rearing rooms, one (a vigorous white-cocoon race called Bagdad) is strongly inclined to normal parthenogenesis, from twenty-five to seventy-five per centum, even in a few cases ninety-five per centum, of the eggs in unfertilized lots beginning to develop. The more usual proportion, however, *i. e.*, that shown by the other races, is, as already noted, less than ten per centum. So much for normal parthenogenesis in the species.

In 1885 Tichomiroff discovered that by bathing the unfertilized eggs with concentrated sulphuric acid, or by rubbing them gently, he could induce a considerably larger per centum than the normal to begin development. He repeated his experiments, confirming and extending his results, in 1902. By histologic examination of the eggs he learned that the artificially stimulated eggs which develop do so in a somewhat abnormal manner. Tichomiroff held the stimulus to development to be neither the action of specific ions, osmotic pressure nor catalysis. He believes that the eggs respond by segmentation to any appropriate excitation, "whatever the nature of this excitation."

Verson, in 1899, used electricity as a stimulus, and found that the development thus initiated ceased at a point about corresponding with that reached by a fertilized egg on the third day after oviposition.

Quajat (1905) submitted unfertilized eggs to the action of oxygen, high temperatures, sulphuric acid, hydrochloric acid, carbon dioxide, and electricity. His account of the experiments indicates that he was able to stimulate development by several of these agents, but he gives no data to show the proportion of developing eggs in the various treated lots. No larva issued, but by an examination of the eggs he found that several embryos had practically completed their development and growth.

My own experiments include the treatment of something over a hundred lots of unfertilized eggs (a "lot" is all the eggs laid by a single female, averaging from 100 to 350 in number), and of several lots of fertilized eggs (to serve as controls to indicate possible injury to the eggs from the reagents used). The stimuli or agents used were dry air (obtained by drawing air through vessels

of calcium chloride and then of concentrated sulphuric acid), high temperature, sunlight, friction, sulphuric acid, hydrochloric acid, glacial phosphoric acid, glacial acetic acid, absolute alcohol, potassium hydroxide, ammonia, and lime water. The reagents were used in different dilutions and for varying lengths of time. The treatment was applied to eggs not more than twelve hours old; mostly to eggs but a few minutes to a few hours old. Five hundred or more lots of untreated, unfertilized eggs were observed in order to determine the extent of normal parthenogenetic development. The eggs of half a dozen silkworm races were used and all the eggs were preserved from time of laying until their death.

As it seemed to me that most of the favorable results obtained by Tichomiroff and Quajat were obtained by treatments which had as common effect a dehydration (such as high temperature, friction, sulphuric acid, etc.) I attempted to test this first by using various dehydrating agents, especially a dry chamber in which the eggs could be submitted for from a minute or two to several hours to a nearly perfectly dry atmosphere. Friction, heat, sulphuric acid, phosphoric pentoxide and glacial phosphoric acid were also used as dehydrating agents. At the same time other treatment, not dehydrating, was used on other lots and gave results hardly less favorable than the dehydrating. The results at the end of this first course of treatment seemed to point to the hydrogen ions as the most likely development-inciting factor. Hence various agents agreeing in containing hydrogen ions though differing radically in other particulars were used. The results gave no encouragement to the hydrogen ion theory. In fact I have not been able to come to an opinion concerning the true *causa efficiens* in the matter. My results simply show to me that various stimuli, acid or alkaline, dehydrating or non-dehydrating, possessing or not possessing hydrogen ions, are able to increase materially the proportion of eggs that develop in lots of unfertilized eggs. The following paragraphs give baldly a summary of the results obtained.

Treatment of Unfertilized Eggs by Dry Air.— Freshly deposited eggs placed in dry chamber for from 14 minutes to 2 hours. Ten lots of unfertilized eggs. In all these lots, except one, a

proportion not exceeding the normal reached the gray stage. In several lots the proportion reaching the cherry (earlier) stage was distinctly above the normal. In one lot two thirds of the eggs reached the gray stage (probably a lot of Bagdad race eggs). One fertilized lot was treated to see if the drying had any injurious effect. Submitted to the dry air for thirty minutes this lot developed normally and all but ten or twelve eggs (a normal number) hatched.

Treatment with Sunlight. — Two lots of unfertilized eggs put in direct sunlight for one and two hours respectively (temperature 35° C.). Ten eggs in each lot reached gray stage, a normal number.

Treatment by Friction. — Several lots rubbed with tooth brush, not very hard. A small increase over normal average of gray eggs, some of these grays persisting alive for nine months, *i. e.*, time for hatching, but none hatched.

Treatment by Heat. — Lots heated in oven to various temperatures from 25° to 57° C. The higher temperatures caused death of all eggs, as well as eggs of fertilized lots used as checks. No increase, over normal average, of developing eggs, through use of the non-fatal temperatures.

Treatment by Phosphoric Pentoxide and Glacial Prosphoric Acid. — Nine lots treated for from one half minute to one hour, the acid applied in some cases as powder, in others as liquid solution. The records are of sufficient interest to give in detail.

Lot 1 : Treatment one hour. Acid put on as powder. Lot of sixty eggs. Treated June 6 (1906).

June 11, three gray eggs.

June 21, six gray eggs.

August 28, twenty-one or more cherry and gray eggs, of which three are alive, others dead.

Lot 2 : Treatment, one hour. Acid put on as powder. Lot of one hundred eggs. Treated June 6 (1906).

June 11, seven gray eggs.

June 21, seven gray eggs.

August 28, thirty cherry and gray eggs (one half are gray), but all are dead.

Lot 3 : Treatment, one hour. Acid put on as powder. Lot of seventy-five eggs. Treated June 6 (1906).

June 11, one gray egg.

June 21, one gray egg.

August 28, twenty-five grayish-pink and five gray eggs, but mostly dead.

March 5 (1907). All eggs are dead.

Lot 4: Treatment, two minutes. Acid in concentrated solution. Lot of sixty eggs. Treated June 20 (1906).

June 27, three eggs, partly gray.

August 28, eight gray; these and most of the yellow eggs still alive.

March 5 (1907), all dead.

Lot 5: Treatment, two minutes. Acid in concentrated solution. Lot of 250 eggs. Treated June 20 (1906).

June 27, five or six gray or cherry eggs.

August 28, fifteen cherry eggs; two gray, a few of which are alive.

March 5 (1907), all dead.

Lot 6: Treatment, one minute. Acid in concentrated solution. Lot of 205 eggs. Treated June 25 (1906).

June 27, one grayish egg.

August 28, three gray, eleven cherry eggs. Almost all alive.

March 5 (1907), all dead.

Lot 7: Treatment, one minute. Acid in solution. Lot of 140 eggs. Treated June 25, (1906).

June 27, two gray eggs.

July 1, fourteen gray eggs, mostly alive. Most of the yellow eggs also alive.

March 5 (1907), all dead.

Lot 8: Treatment, one half minute. Acid in solution. Lot of fifty eggs. Treated June 27 (1906).

July 1, two cherry eggs.

August 28, five gray eggs; several cherry, mostly alive. About two thirds of the yellow eggs also alive.

Lot 9: Treatment, one half minute. Acid in solution. Lot of ninety eggs. Treated June 27 (1906).

July 1, eight cherry or grayish eggs.

August 28, twenty-seven gray or pink-gray eggs of which

only two or three are collapsed Of the sixty or more yellow eggs, only six or seven are collapsed.

March 5 (1907), four live gray eggs; all others dead.

The treatment with glacial phosphoric acid seems to have the curious effect of prolonging the life of all the eggs whether they begin actual development or not, and of *slowly* initiating development in a considerable fraction of them, a proportion distinctly above the average number that would begin development without artificial stimulus.

Treatment by Sulphuric Acid. — Sixteen lots of unfertilized eggs and two of fertilized (as controls to indicate possible injury by the reagent) were treated with concentrated sulphuric acid for periods varying from one fourth of a minute to two minutes, and then washed with water. This acid is, of course, a strong dehydrator. In several cases only part of a lot would be treated, the other part left untreated as a check lot. The fertilized eggs developed normally and hatched, showing that the concentrated acid applied for two minutes does not injure the eggs. In all the treated unfertilized lots the proportion, above the normal average, of developing eggs was materially increased. This is also true of the treated parts of lots as compared with the untreated.

For example, in lot 2, a large lot of four hundred and fifty eggs, one hundred and fifty were treated and three hundred left untreated. In seven days ninety of the treated eggs were gray, while only five of the untreated eggs were gray. In lot 3, one hundred and forty eggs, one hundred were treated and forty left untreated. In ten days more than half the treated eggs were gray and alive, while none was gray in the untreated part. On the average from thirty to fifty per centum of the eggs in treated lots or fractions of lots began to develop, while in untreated parts of lots the per centum of developing eggs was less than ten.

Treatment by Hydrochloric Acid. — Twenty-four lots of unfertilized eggs treated with concentrated hydrochloric acid or with ten per centum hydrochloric acid, for periods of from one fourth minute to two minutes, then washed with water. The acid has but little dehydrating effect. On the whole the results show the distinctly stimulating effect of the acid, but some lots behaved aberrantly and the proportion of developing eggs did not go

beyond thirty per centum, and was usually not more than twenty or twenty-five per centum. The eggs treated with concentrated acids for the shorter periods, *i. e.*, one fourth and one half minute, were in better condition than those treated for one or two minutes. The eggs treated with ten per centum hydrochloric acid showed no special stimulation.

Treatment with Absolute Alcohol. — Killed the eggs.

Treatment with Potassium Hydroxide — Eleven lots of unfertilized eggs treated with strong solution of potassium hydroxide for periods ranging from one fourth minute to two minutes. All the eggs treated were loosened from their resting place and soon collapsed. Before dying the eggs showed a reddish color like the normal cherry of developing eggs, but from the great prevalence of this color in all the treated lots and parts of lots I am inclined to believe this color due to some special effect of the reagent on the egg shell rather than the indication of development. In the lots treated with strong solution for two minutes death and collapsing soon occurred, and in the lots and parts of lots treated for one fourth minute with half strength solution, collapsing occurred before it did in untreated lots and parts of lots.

Treatment with Lime Water. — Five lots of unfertilized eggs were treated with saturated lime water for periods varying from three minutes to one hour. No increase in proportion of developing eggs. The eggs of a fertilized lot treated with lime water for three minutes; all (except the small normal per centum) developed and hatched.

Treatment with Glacial Acetic Acid. — Seven unfertilized lots treated with glacial acetic acid, strong and half strong, for periods of one minute. Behavior of the lots uneven. In three of the lots no stimulating effect was noticeable. In two about ten per centum of the eggs developed. In one about thirty per centum began development, while in lot 7, a lot of three hundred and twenty-five eggs, half of which were treated and half left untreated more than fifty per centum of the treated eggs began development, while in the untreated lot very few, not more than two per centum. In a fertilized lot treated with the acid for one minute, all the eggs, except four or five, developed and hatched.

Treatment with Ammonium Hydroxide. — Six unfertilized lots

treated with strong ammonium hydrate for periods of one half minute or one minute. In two lots there was a beginning development of one third of the eggs ; in the other four lots no increase over the normal average. In one of the two lots showing stimulation some of the eggs were left untreated and the increase in proportion (reaching thirty-three per centum) of the developing eggs occurred only in the treated portion of the lot. In the untreated portion only four per centum of the eggs began development. A fertilized lot treated with the reagent developed and hatched normally.

STANFORD UNIVERSITY, CALIF.